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Impact of dietary protein supplements on the economic traits of Mulberry Silkworm (*Bombyx mori* L.)

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Abstract
The mulberry silkworm (*Bombyx mori* L.) is an important model insect in nutritional and developmental studies, with its growth and reproduction strongly dependent on dietary protein. The present study, conducted during March-April 2025 at the Experiential Learning Programme Unit on Commercial Sericulture, College of Agriculture, Latur (VNMKV, Parbhani), evaluated the impact of protein-fortified mulberry leaves on the biological performance of the bivoltine double hybrid CSR2 reared on V-1 mulberry variety under a Completely Randomized Design. Results showed that supplementing mulberry leaves with plant-based protein sources significantly improved larval and economic traits compared to the control. Among all treatments, 0.5% soya flour enrichment produced the best outcomes, including the lowest disease incidence (1.50%), the highest mature larval weight (47.23 g/10 larvae), maximum effective rate of rearing (97.03%), and highest cocoon yield (20.70 kg per 10,000 larvae). In contrast, larvae fed distilled water–treated leaves showed the poorest performance, with the lowest larval weight (39.57 g), lowest ERR (90.40%), lowest cocoon yield (14.83 kg), and the highest disease incidence (5.73%). Overall, the study confirms that protein supplementation through soya, groundnut, pigeon pea, pea, gram, black gram, and green gram flours enhances larval growth, cocoon production, and overall silkworm performance. The findings suggest that dietary proteins may directly influence reproductive physiology, potentially by improving vitellogenin synthesis and other key biochemical processes. Soya flour at 0.5% emerged as the most effective supplement, while untreated controls consistently underperformed, highlighting the critical role of protein-rich diets in silkworm development and productivity.

Keywords: Bombyx mori, dietary proteins, effective rate of rearing, disease incidence, nutritional physiology

Introduction

Silkworm (*B. mori*) is a monophagous lepidopteron insect that is reared for silk production under a special controlled environment (Fambayun *et al.* 2022) [5]. Silkworm derives all essential nutrients from mulberry leaf for growth and development (Chanotra, and Bhat 2021) [4]. The growth and development of silkworm larvae is greatly influenced by the mulberry leaves' quality (Prasad and Madhavi 2020; Murugesh *et al*. 2022; Saha *et al*. 2022) [17, 15, 21]. The production of cocoons is also affected by different factors including environmental conditions, planting and pruning and soil characteristics that affect mulberry leaf quality (Samami *et al.* 2019) [22]. The dietary and nutritional requirements of the silkworm including water, vitamins, carbohydrates, proteins, fats and ascorbic acid are met by digestion and assimilation of mulberry leaves (He *et al* 2021; Rajan *et al.* 2022) [8, 19]. Furthermore, proteins present in mulberry leaves contribute to more than 70% of silk synthesis in silkworms, thereby playing a crucial role in determining cocoon yield. (Alipanah *et al*. 2020). Nutritional supplementation plays a crucial role in improving the growth and productivity of silkworms. Additives enriched with vitamins, amino acids, and proteins have been reported to significantly enhance the economic traits of *Bombyx mori* (Amalarani *et al*., 2011) [2]. Soy protein, being a rich dietary source, has been recognized for its role in enhancing larval growth and improving the economic traits of *Bombyx mori*.

Daily supplementation of soy protein significantly contributes to increased quality and quantity of silk cocoon production (Ito, 1980; Kamaraj *et al*., 2017) [10, 11]. Larvae of *B. mori* have been successfully reared on semi-artificial diets supplemented with various protein sources such as soybean, mushroom, corn flour, and their mixtures. Among these, corn flour-based diets during the fifth instar recorded the highest values for larval duration, larval weight, silk gland weight, pupal weight, cocoon and shell weight, as well as the number of eggs deposited, while also resulting in the lowest mortality percentage (Mona Mahmoud, 2013) [11].
The growth and development of silkworms are strongly influenced by the nutritional quality of mulberry leaves (Masthan *et al*., 2011) [14]. This nutritional value can be further enhanced through enrichment with supplementary nutrients. Studies have demonstrated that honey supplementation improves both biological performance and economic traits of *Bombyx mori* (Saad *et al.*, 2014; Thulasi and Siva Prasad, 2015; Alagumanikumaran and Prema, 2016; Gad, 2013) [20, 23, 1, 6]. Protein-rich food sources are recognized for enhancing the economic traits and supporting the growth of silkworms, with soy protein being a notable example (Rahman, 2018) [18]. *Bombyx mori* utilizes all amino acids in egg albumen, especially the essentials during growth and development, to synthesize silk fiber (Islam *et al*., 2020) [9]. To assess this, the effect of different dietary protein-treated mulberry leaves was investigated on the life cycle of the mulberry silkworm (*Bombyx mori* L.).

Material and Methods / Experimental Details / Methodology

The study was conducted during March–April 2025 at the Experiential Learning Programme Unit, Department of Agricultural Entomology, College of Agriculture, Latur, Maharashtra, to evaluate the effect of protein-treated mulberry leaves on economic traits of *Bombyx mori*.

Materials

Disease-free layings (DFLs) of mulberry silkworm were fed on mulberry variety V-1 leaves sourced from the Instructional-cum-Research Farm, Department of Agricultural Entomology, College of Agriculture, Latur. Essential equipment included plastic rearing trays (36” × 24” × 3”), iron stands, chopping boards, knives, bamboo sticks, feathers, cleaning nets, collapsible plastic mountages (2 × 0.95 m²), paraffin papers, foam pads, and a digital electronic balance for weighing larvae, pupae, cocoons, and shells.

Experimental treatments

Eight treatments involved mulberry leaves sprayed with 0.5% protein supplements derived from soya, groundnut, pigeon pea, pea, gram, black gram, green gram flours, and distilled water as a control. Leaves were sprayed, air-dried, and fed to larvae until pupation.

Rearing method

DFLs of CSR2 bivoltine double hybrid silkworms were obtained from SSPC, Latur. Eggs at the blue stage were incubated in black boxes to ensure uniform hatching. After hatching, 100 chawki larvae per treatment per replication were transferred to separate trays. Early instars were fed tender mulberry leaves, while later instars received mature leaves treated with dietary proteins. Bed size, spacing, and feeding schedules followed standard protocols (Krishnaswami, 1978; Nalwandikar *et al.*, 2017) [12, 16].
Environmental conditions were maintained at 22–28 °C temperature, 60–90% relative humidity, with 14–16 hours light and 8–10 hours darkness, monitored using thermo-hygrographs. Rearing trays were disinfected using 2% formalin and 0.3% lime powder before, during, and after rearing. Leaves were chopped according to larval preferences and fed at 6, 10, 16, and 21 hours daily. Larvae were not disturbed during moulting; beds were dusted with Vijetha (4 kg/100 DFLs) post-moulting and cleaned regularly. Feed quantity was adjusted as larvae grew.
Cocoons were harvested on the fifth day. Three random samples of 10 cocoons per treatment were selected to measure cocoon weight, filament length, and moth emergence.

Observations Recorded:

Disease Incidence (%)

Grasserie- and flacherie-infected larvae were counted, and disease incidence was calculated as:
Disease Incidence (%) = Diseased larvae ÷ Total larvae×100

Effective rate of rearing (%)

ERR was computed using:
ERR (%) = No. of cocoon harvested ÷ No. of larvae retained x 100

Cocoon Yield (kg/10,000 larvae)

One hundred cocoons were randomly weighed, and yield per 10,000 larvae brushed was estimated.

Larval Weight (g)

The weight of 10 matured larvae was recorded and expressed in grams.

Statistical analysis

Data on biological and economic traits were subjected to analysis of variance (ANOVA) using OPSTAT software. Differences among treatments were tested for significance at the 5% probability level by the F-test, following the procedures described by Gomez and Gomez (1984).

Experimental design

The experiment was arranged in a Completely Randomized Design (CRD) with eight treatments and three replications. Each treatment involved 100 silkworms per replication.

Results and Discussion

Table 1: Effect of different dietary protein treated mulberry leaves on disease incidence, larval weight, effective rate of rearing and cocoon yield per 1000 larvae of mulberry silkworm

Tr. no.	Treatment details	Disease incidence (%)	Mean Larval Weight (g)	Mean (ERR)	Mean cocoon yield/10000 larvae (kg)
T_1	Mulberry leaves treated with protein @ 0.5 per cent of Soya Flour	1.50 (7.03) *	47.23	97.03 (80.23) *	20.70
T_2	Mulberry leaves treated with protein @ 0.5 per cent of Ground Nut Flour	2.27 (8.65) *	46.40	96.10 (78.81) *	18.83
T_3	Mulberry leaves treated with protein @ 0.5 per cent of Pigeon Pea Flour	3.60 (10.93) *	42.30	94.40 (76.56) *	17.27
T_4	Mulberry leaves treated with protein @ 0.5 per cent of Pea Flour	4.43 (12.15) *	41.10	91.33 (72.99) *	15.83
T_5	Mulberry leaves treated with protein @ 0.5 per cent of Gram Flour	4.03 (11.58) *	43.30	92.60 (74.45) *	16.43
T_6	Mulberry leaves treated with protein @ 0.5 per cent of Black Gram Flour	3.23 (10.35) *	44.97	93.23 (74.92) *	17.83
T_7	Mulberry leaves treated with protein @ 0.5 per cent of Green Gram Flour	2.93 (9.86) *	45.33	95.53 (77.99) *	18.13
T_8	Mulberry leaves treated with distilled water	5.73 (13.84) *	39.57	90.40 (71.96) *	14.83
	C.D.	0.23	1.86	3.94	1.27
	S.E. (m)	0.07	0.62	1.31	0.42
	S.E. (d)	0.11	0.87	1.85	0.59
	C.V. (%)	3.87	2.43	2.41	4.16

*Figures in parenthesis are mean arcsine transformed values

Effect of different dietary protein treated mulberry leaves on disease incidence of mulberry silkworm

The effect of different dietary protein-treated mulberry leaves on disease incidence in mulberry silkworms is presented in Table 1. Disease incidence across treatments ranged from 1.50 to 5.73%. The lowest incidence (1.50%) was recorded in treatments with 0.5% soya flour, indicating superior nutrition and better disease resistance. Moderate disease levels were observed in treatments with 0.5% groundnut flour (2.27%), green gram flour (2.93%), and black gram flour (3.23%). Slightly higher incidences occurred with pigeon pea flour (3.60%), gram flour (4.03%), and pea flour (4.43%). The highest disease incidence (5.73%) was recorded in the control, where larvae were fed leaves treated only with distilled water. The results are accordance with of Jaybhay (2018) reported that the effect of dietary supplement treated mulberry leaves on disease incidence of mulberry silkworm revealed that none of the treatment evidenced disease occurrence. Whereas, Hamzah Mohamed Kamel (2014) showed that feeding of larvae on treated leaves lead to decreased larval mortality percentages, supporting the findings of present study.

Effect of different dietary protein treated mulberry leaves on weight of matured larvae of mulberry silkworm

The effect of protein-treated mulberry leaves on mean larval weight is shown in Table 1. The mature larval weight ranged from 39.57 to 47.23 g. The highest weight (47.23 g) was obtained with leaves treated with 0.5% soya flour, which was statistically at par with groundnut flour (46.40 g) and followed by green gram flour (45.33 g). Moderate weights were recorded with black gram (44.97 g), gram flour (43.30 g), and pigeon pea flour (42.30 g). Pea flour resulted in 41.10 g, while the lowest weight (39.57 g) occurred in the control. These results show that dietary proteins play a key role in larval growth and development. Maqbool *et al*. (2023) reported that mulberry leaves supplemented with spirulina and thyroxine improved cocoon yield. In spring, the combination of spirulina (500 ppm) and thyroxine (5 ppm) produced the highest cocoon yield (21.44 kg/10,000 larvae), while the control without treatment recorded the lowest (15.77 kg). Similarly, in autumn, the same combination achieved the maximum yield (18.54 kg), whereas the untreated control showed the minimum (12.64 kg/10,000 larvae). However, Kamaraj *et al*. (2017) [11] who indicated that among the treatments, soya solution treated leaves observed higher larval weight of silkworm as compared to other treatments. Also, Krishnan *et al*. (1995), they showed that the hydrolyzed soya protein (P-soyatase) supplementation decreased the larval duration and increased the accumulation of haemolymph protein (SP-1: female specific protein and SP-2; an arlyphorin), larval weight.

Effect of different dietary protein treated mulberry leaves on effective rate of rearing (ERR) of mulberry silkworm

The effect of protein-treated mulberry leaves on the effective rate of rearing (ERR) is shown in Table 1. ERR ranged from 90.40% to 97.03%. The highest ERR (97.03%) was obtained with leaves treated with 0.5% soya flour, which was at par with groundnut flour (96.10%), followed by green gram flour (95.53%) and pigeon pea flour (94.40%). Moderate ERR values were recorded with black gram flour (93.23%), gram flour (92.60%), and pea flour (91.33%). The lowest ERR (90.40%) occurred in the control treatment. These findings align with earlier studies. Shruti *et al*. (2019) reported the highest ERR in silkworms fed azolla-supplemented leaves (95.73% in June–July and 92.87% in March–April), followed by the control and soy milk treatments. Rahul *et al*. (2017) found no significant difference in ERR when larvae were fed leaves enriched with 0.5–1% yeast extract. noted that enriching leaves with asparagine (0.1%) and alanine (0.5%) improved ERR, while recorded the highest ERR with alanine-enriched leaves (1000 ppm).

Effect of different dietary protein treated mulberry leaves on cocoon yield per 10,000 larvae brushed of mulberry silkworm

The effect of protein-treated mulberry leaves on cocoon yield per 10,000 larvae is shown in Table 1. Cocoon yield ranged from 14.83 to 20.70 kg. The highest yield (20.70 kg) was obtained with leaves treated with 0.5% soya flour, significantly outperforming all other treatments. Groundnut flour (18.83 kg) and green gram flour (18.13 kg) produced comparable yields, followed by black gram flour (17.83 kg). Moderate yields were recorded with pigeon pea flour (17.27 kg) and gram flour (16.43 kg), which were at par. Pea flour resulted in 15.83 kg, while the lowest yield (14.83 kg) occurred in the control.

Conclusion

The overall results indicate that fortifying mulberry leaves with protein supplements specifically soya flour, groundnut flour, pigeon pea flour, green gram flour, gram flour, and black gram flour positively influences the growth and development of *Bombyx mori* larvae, as well as cocoon and silk production. This enhancement is likely attributable to increased protein availability during the larval stages, which significantly affects subsequent pupal and adult stages. Among the treatments, mulberry leaves enriched with 0.5% soya flour yielded the most favourable outcomes, including reduced larval and pupal duration, higher moth emergence, increased fecundity and hatching rates, improved effective rate of rearing, and superior cocoon and silk quality parameters such as single cocoon weight, cocoon yield, filament length, filament weight, and denier. These findings confirm soya flour as the optimal protein supplement for sericulture applications. Therefore, the application of mulberry leaves treated with 0.5% soya flour is strongly recommended as a dietary supplement to enhance the biological performance of *B. mori*.

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